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Fluorometric Determination of Magnesium in Soil Extracts

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FLUOROMETRIC DETERMINATION OF MAGNESIUM IN SOIL EXTRACTS

BY

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IN SOIL EXTRACTS

This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable as meeting the thesis requirements for this degree, but without implying that the conclusions reached by the candidate are necessarily the conclusions of the major department.

Thesis Adviser — Date

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INTRODUCTION

Determination of magnesium in soils has been studied extensively in the last half-century. The classical gravimetric method, based on precipitation of magnesium ammonium phosphate, has been replaced largely by faster and more convenient methods. Volumetric methods involving titration of Mg with various indicators were the first major changes. With advanced instrumentation, spectrophotometric and flame photometric methods have been used widely. More recently atomic absorption spectrophotometry has been used to determine Mg in soil extracts.

All procedures mentioned have various disadvantages including color stability, foreign ion interference, equipment cost, and convenience. There was a need for a reliable, economical, and convenient method for determination of Mg in soil extracts.

The purpose of this study was to examine fluorescence analysis as a fast and reliable method for determination of Mg in soil extracts.

LITERATURE REVIEW

In the search for a rapid and reliable test for Mg in soil extracts, many different approaches have been made. Alexander and Harper (1) studied the determination of exchangeable Mg in soil by precipitation as 8-hydroxyquinoline. A colorimetric procedure detecting magnesium quinolate dissolved in hydrochloric acid was recommended as a rapid and accurate method for the determination of small quantities of Mg.

Peech (12) proposed the titan yellow method for determination of exchangeable Mg to correlate with the occurrence of Mg deficiency symptoms in citrus. He concluded that because of its simplicity and rapidity, the titan yellow method was suitable for routine soil testing but was not quantitative enough for analytical work of greater accuracy.

Because of difficulty in obtaining superior German-made titan yellow during World War II, Mikkelsen and Toth (9) made a study to find a substitute. Thiazol yellow (sodium 2-2 disulfonate of methylbenzolthiazole) because of its greater and broader range of sensitivity, was found to be by far the best of all dyes tested.

Colorimetric determinations of Mg by colored lake reactions were modified several times (7, 10, 13, 22). These determinations were subject to fundamental difficulties which resulted in errors due to inconsistent color intensities and difficulty in measuring the color.

The presence of other substances may have affected the complex or formed precipitates in the alkaline medium. In methods proposed by Yien and Chesnin (21) titan yellow and thiazole yellow were used for analysis of Mg in plant and soil extracts. In these methods, errors due to varying or inconsistent color intensities were controlled. A sodium bisulfite solution was used to inhibit oxidation of dyes, stabilize the colored lake and reduce fading. Suspension stability was obtained with a starch suspension in the titan yellow procedure, and with polyvinyl alcohol in the thiazole yellow procedure.

Cheng and Bray (4) reported on determination of Ca and Mg by titration with EDTA (disodium-dihydrogen ethylenediaminetetracetate) using Murexide and Eriochrome Black T as indicators. A comparison was made by Tucker and Kurtz (18) of the above method with three other methods for the determination of Mg by titration with EDTA. It was concluded that the Cheng and Bray procedure was extremely useful for determinations of exchangeable Ca and Mg in most soils. However, errors accumulated in the Mg determination since it was estimated by difference, especially when the concentration of Mg was low in proportion to Ca. Overall, procedures for determining exchangeable Ca and Mg by titration with EDTA were sufficiently sensitive, but suffered from the indistinct nature of the end-point, the necessity of determining Mg by difference and the possibility of interference from aluminum and iron.

Application of the Beckman flame photometer to determination of Mg in soil extracts was initially studied by Rich (14). Analysis of Mg by flame emission was found to be more difficult than analysis of other cations because of the higher excitation potential of the element, and intense flame background in the region of the Mg spectrum. Shaw and Veal (17) continued the study of determining exchangeable Ca and Mg with flame emission. Their study was of instrumental adjustments and associated ion interferences. Shaw (16) later reported on a collaborative study of methods for determination of exchangeable Mg in soil extracts. Solutions of Mg and Ca in ammonium acetate were prepared and sent to several state and federal laboratories to compare their methods. Five different methods were used; thiazole yellow colorimetry, EDTA titration, magnesium ammonium phosphate titration, pyrophosphate gravimetric analysis, and flame photometry. Results were summarized as follows: (a) Thiazole yellow gave average results closer to the true values than other methods, (b) Flame photometry methods gave consistently low results, (c) EDTA titration gave high results and agreement among collaborators was low, (d) Magnesium ammonium phosphate titration over-estimated Mg and collaborator agreement was poor. The study was continued to compare relative precisions of flame photometry and thiazole yellow. It became evident early in this study that the colorimetric procedure was not satisfactory. Some collaborators failed to report their results and others expressed skepticism in their results because of large

differences among replicates. Attoe (3) continued the report on the collaborative study of flame photometric determination of exchangeable Mg in soils. Soil samples ranging in exchangeable Mg from about 40 to 1,600 ppm. were sent to collaborating laboratories. The samples were extracted with ammonium acetate and Mg was determined using the Beckman Model DU flame photometer. The same samples were tested with the standard AOAC method (2) and comparisons of results were made. In every case, average values for the flame photometric determination were lower than those for the standard AOAC method.

David (5) examined atomic absorption spectrophotometry for determination of exchangeable Mg in soils. Strontium chloride was added to solutions to cover interferences from phosphate, aluminum, silicate, and sulfate. Results obtained were in good agreement with standard methods. However, the need for elaborate and expensive equipment was a disadvantage with the atomic-absorption method. Instrument instability necessitating frequent readings of standards, and lengthy calculations in converting per cent absorption to Mg concentration were other undesirable factors.

Review of the many proposed methods to determine Mg in soil extracts indicated a simple and reliable method was still needed.

In a study of the fluorescence of several azo compounds known to form Ca and Mg derivatives, Olsen and Diehl (11) found that o,o'-dihydroxyazobenzene combined with Mg to form a highly fluorescent derivative. The azo compound alone or in the presence of Ca displayed

no, or very weak fluorescence. Diehl et al. (6) used this azo compound to develop a fluorometric method for determination of Mg. The method has been applied to determination of Mg in water, limestone, and serum. The results of these studies stimulated an investigation using o,o'-dihydroxyazobenzene as a fluorometric reagent for determination of Mg in soil extracts.

THEORY

Fluorescence Analysis

As with many other analytical techniques, application of fluoresence assay does not require knowledge of the theoretical background of the phenomenon. However, some understanding of physical principles of fluorescence is helpful in extending its use.

Certain compounds become electronically excited by irradiation with high-energy photons. The molecules absorb photons of light, raising low energy electrons to higher energy levels. Then, the molecules are in an excited state. If these molecules do not decompose as a result of the increase in energy and if all the energy is not dissipated by subsequent collisions with other molecules, then after a short period of time (10^{-8} to 10^{-7} second), the electrons return to the lower energy level, emitting photons. Because some energy is dissipated in establishing the higher energy level, the photons subsequently emitted are of lower energy and longer wavelength than those absorbed. This radiation of lower energy is called fluorescence (19).

Many compounds combine with metals to form fluorescent complexes. Because fluorescence is directly proportional to the concentration of the complex, a measurement of the fluorescence would be a measurement of the metal concentration.

EXPERIMENTAL METHODS AND PROCEDURES

The Absorption and Fluorescence Spectra

Absorption spectra for 5×10^{-5} M o,o'-dihydroxyazobenzene and 5×10^{-5} M magnesium-o,o'-dihydroxyazobenzene in 95% ethanol were determined with a Beckman Model DK-2A spectrophotometer. Fluorescence spectra of the same solutions were obtained on the same instrument with the Beckman Model No. 73500 fluorescence attachment. The only alteration was to replace the standard equipment F4T5/BL lamp and Schott UG 11 filter with the F4T5/BL lamp and Corning CS-5-60 glass color filter. The alternate lamp and filter provided a band of light for excitation which corresponded more closely to the absorption spectrum of the magnesium-azo complex.

Selection and Characterization of Soil Samples

Samples of eight surface soils were selected for testing the fluorometric determination of Mg. The soils selected were from the Great Soil Groups: Grey Brown Podzolic, Brunizem, Chernozem, and Chestnut. The samples were air dried, passed through a 2-mm. sieve, and stored in polyethylene bags. The air dry samples were studied with routine laboratory analysis to obtain some information about the soils. The cation exchange capacity was characterized according to Russel (15) with ammonium acetate. The ammonium acetate extracts were collected and exchangeable Mg and Ca determined with atomic absorption

spectrophotometry according to the method of David (5). A variation from the method proposed was use of ammonium acetate rather than ammonium chloride as the extracting reagent. Lanthanum was used to cover interferences rather than strontium in the Ca determination. The Perkin-Elmer Model 303 atomic absorption spectrophotometer was used to make absorption measurements.

The alkaline earth carbonates, pH of the saturated soil paste, and electrical conductivity of the saturation extract were determined as suggested in the U.S.D.A. Agricultural Handbook No. 60 (20).

Preparation of Soil Extracts

Magnesium concentrations were determined both in saturation extracts and ammonium acetate extracts of the soil samples. Saturation extracts were obtained by saturating 100 grams of air dry sample with water as described in U.S.D.A. Agricultural Handbook No. 60 (20). After one hour, the extract was removed by placing the soil paste in a suction filter for fifteen minutes.

Exchangeable Mg was determined in neutral normal ammonium acetate soil extracts. The extracts were prepared according to Russel (15). Five grams of air dry sample were shaken with 25.0 ml. of ammonium acetate. After one-half hour, the mixture was filtered and the soil washed with another 25.0 ml. of ammonium acetate. The

filtrate and washing were collected and diluted to the mark in a 100 ml. volumetric flask with ammonium acetate. Small amounts of ammonium acetate did not interfere with the fluorometric determination of Mg. Therefore, the same procedure was used to determine Mg in saturation and ammonium acetate extracts.

Optimum Concentration Range for Calibration Curve

The Mg concentration range where best results of fluorescence analysis could be expected was studied. Magnesium solutions varying widely in concentration were prepared and fluorescence intensity was measured. A standard calibration curve was determined in this concentration range.

Interferences of Foreign Ions

Several experiments were performed testing the effect of foreign ions on the fluorometric determination of Mg. Magnesium standards were prepared with additions of foreign ions approximating their concentration in soil extracts. The ions tested were Ca^{+2}, Al^{+3}, PO_4^{-3}, Mn^{+2}, Na^{+1}, Cu^{+2}, SO_4^{-2}, Cl^{-1}, and NO_3^{-1}.

The experiments were extended to determine the extent of interference by Ca. Saturation extracts were prepared for fluorescence analysis with two levels of Ca added to each sample. Standard Ca solution was prepared with reagent-grade calcium carbonate and aliquots of this solution added to the samples to be equivalent to additions of 75 and 150 ppm. Ca to the extracts. Additions of 75 and 150 ppm. Ca

were approximately equal to the range of Ca concentrations found in the saturation extracts. Fluorescence of each solution was measured and the Mg concentration determined with a standard calibration curve. Results of the samples with Ca added were compared to those with no Ca added.

To help evaluate the interference of added Ca, the Ca concentration of the original saturation extracts was determined. The method of David (5) was used making solutions up to volume in water.

Because of small positive error in the Mg determination when Ca was added, the experiments were repeated. A new working solution was prepared containing Ca. The addition of this working solution (II) to samples and standards added Ca equivalent to 500 ppm. Fluorescence of all solutions was measured and Mg concentration determined with the calibration curve prepared with this working solution (II). Results of the samples with 75 and 150 ppm. Ca added were compared to similar samples with no Ca added.

Fluorometric Determination of Magnesium

Instrumentation

A Turner Associates Model 110 fluorometer was used for fluorescence measurements in this study. The standard general purpose light source was replaced with a Turner Accessory #110-853 blue lamp. The alternate blue lamp provided greater light intensity at the wavelength for maximum excitation of the magnesium-o,o'-dihydroxyazobenzene complex. Corning glass color filters CS-5-60 and CS-3-67 were used in

the excitation and fluorescent beams respectively. The range selector which controlled the amount of light striking the samples was set at 3X for all fluorescence measurements.

Reagent Preparation

(a) Stock Solution of 2.5×10^{-3} M o,o'-Dihydroxyazobenzene, Diehl et al. (6). One liter was prepared by dissolving 0.5355 gram of crystalline o,o'-dihydroxyazobenzene[1] in a mixture of 10 ml. ethanol and 10 ml. 2M potassium hydroxide. Water was added to complete the solution which was stored in a polyethylene container.

(b) Working Solution (I) of 7.15×10^{-5} M o,o'-Dihydroxyazobenzene, Diehl et al. (6). One hundred ml. of 95% ethanol and 19 ml. of anhydrous ethylenediamine were placed in a 1-liter volumetric flask; mixed and allowed to cool. Five ml. of a mixture of equal parts by volume of concentrated hydrochloric acid and water were added slowly. The solution was again mixed and cooled. Next were added 14.3 ml. of triethanolamine and 28.6 ml. of o,o'-dihydroxyazobenzene stock solution. The solution was diluted to 1-liter with 95% ethanol, mixed and stored in a polyethylene container.

(c) Working Solution (II) of 7.15×10^{-5} M o,o'-Dihydroxyazobenzene and 5.57×10^{-5} M Ca. One hundred ml. of 95% ethanol and 19 ml. of anhydrous ethylenediamine were placed in a 1-liter volumetric flask; mixed and allowed to cool. Five ml. of a mixture of equal parts by

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[1] This reagent was obtained from the G. Frederick Smith Chemical Co., Columbus, Ohio.

volume of concentrated hydrochloric acid and water were added slowly. The solution was again mixed and cooled. Then 14.3 ml. of triethanolamine and 28.6 ml. of o,o'-dihydroxyazobenzene stock solution was added. Exactly 0.0056 gram reagent-grade calcium carbonate was added. The solution was diluted to 1-liter with 95% ethanol; mixed and stored in a polyethylene container.

(d) Standard Solution of $1x10^{-2}$ M Mg. Exactly 0.2400 gram Grignard-grade Mg metal was dissolved in a minimum of hydrochloric acid, diluted to 1-liter and mixed thoroughly. A secondary Mg standard ($6x10^{-5}$ M) was prepared by pipetting 6 ml. of the $1x10^{-2}$ Mg standard solution into a 1-liter volumetric flask; diluting to the mark and mixing.

Analytical Procedure

Magnesium concentrations in saturation and ammonium acetate soil extracts were determined by the following procedure.

Exactly 0.100 ml. of extract was transferred to a 50 ml. volumetric flask. Thirty five ml. of working solution (I) were added with an automatic pipette.[2] The mixture was diluted to the mark with water and mixed thoroughly.

Magnesium standards were prepared by transferring aliquots of the $6x10^{-5}$ M Mg into 50 ml. volumetric flasks. The range of standards

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[2]The automatic pipette, K-76338 35.0 ml., was obtained from Kontes Glass Co., Vineland, New Jersey.

used was 0.6 to 3.6×10^{-6} M Mg. The standards were mixed with 35.0 ml. of working solution (I) and diluted to volume with water.

Fluorescence of the standards and samples was measured in the Turner fluorometer. The instrument was set at zero fluorescence and the galvanometer brought to its null point against a blank prepared of 35.0 ml. of working solution (I) and 15.0 ml. water.

A calibration curve was prepared by plotting relative fluorescence intensity versus concentration of the Mg standards. The calibration curve was used to determine Mg concentration in the samples.

Recovery Experiments

As a means of testing the accuracy of the method, a number of determinations were made on the recovery of Mg added to soil extracts. Saturation extracts were prepared for fluorescence measurements with two different levels of Mg concentration added. A standard Mg solution was prepared from Mg metal and aliquots of this solution added to the samples to be equivalent to an addition of 5 and 10 ppm. Mg to the extracts. Fluorescence of each solution was measured and the Mg concentration determined with a standard calibration curve. Results of the samples with Mg added were compared to similar samples with no Mg added.

Comparison with Other Methods

Results of the fluorometric method were compared with the results obtained by two other methods. Magnesium was determined in

saturation extracts by atomic absorption according to David (5). A variation from the procedure was to make solutions up to volume in water, rather than ammonium chloride. Magnesium was determined in the ammonium acetate extracts by the same method making solutions to volume in ammonium acetate.

Magnesium concentrations also were determined with the thiazol yellow colorimetric method of Graham (8). The Beckman Model DU spectrophotometer was used to make absorption measurements.

RESULTS AND DISCUSSION

The Absorption and Fluorescence Spectra

Maximum sensitivity in fluorescence analysis requires selection and measurement of light at the wavelength of maximum fluorescence. Because fluorescence intensity from a compound depends on its degree of excitation, the wavelength of light which induces maximum excitation must be known. In theory, the excitation spectrum should be identical to the absorption spectrum. Therefore, the wavelength of maximum excitation should correspond to the wavelength of maximum absorption. Because of instrument limitations and for convenience, the absorption spectra were determined. The absorption and fluorescence spectra of o,o'-dihydroxyazobenzene and the magnesium-o,o'-dihydroxyazobenzene complex are shown in Figure 1. After the spectral characteristics of Mg-azo complex were determined, correct selection of a light source, filters, and detector was made.

Absorption and fluorescence spectra showed maximum excitation occurred at a wavelength of 470 mu and the maximum fluorescence at a wavelength of 580 mu. Therefore, for maximum excitation, the Turner Accessory #110-853 blue lamp was used to provide maximum light intensity at 470 mu. A primary filter was selected to allow light of 470 mu wavelength to strike the sample and a secondary filter to allow the fluoresing light at 580 mu wavelength to reach the detector. Filters selected for this determination were Corning glass color filters,

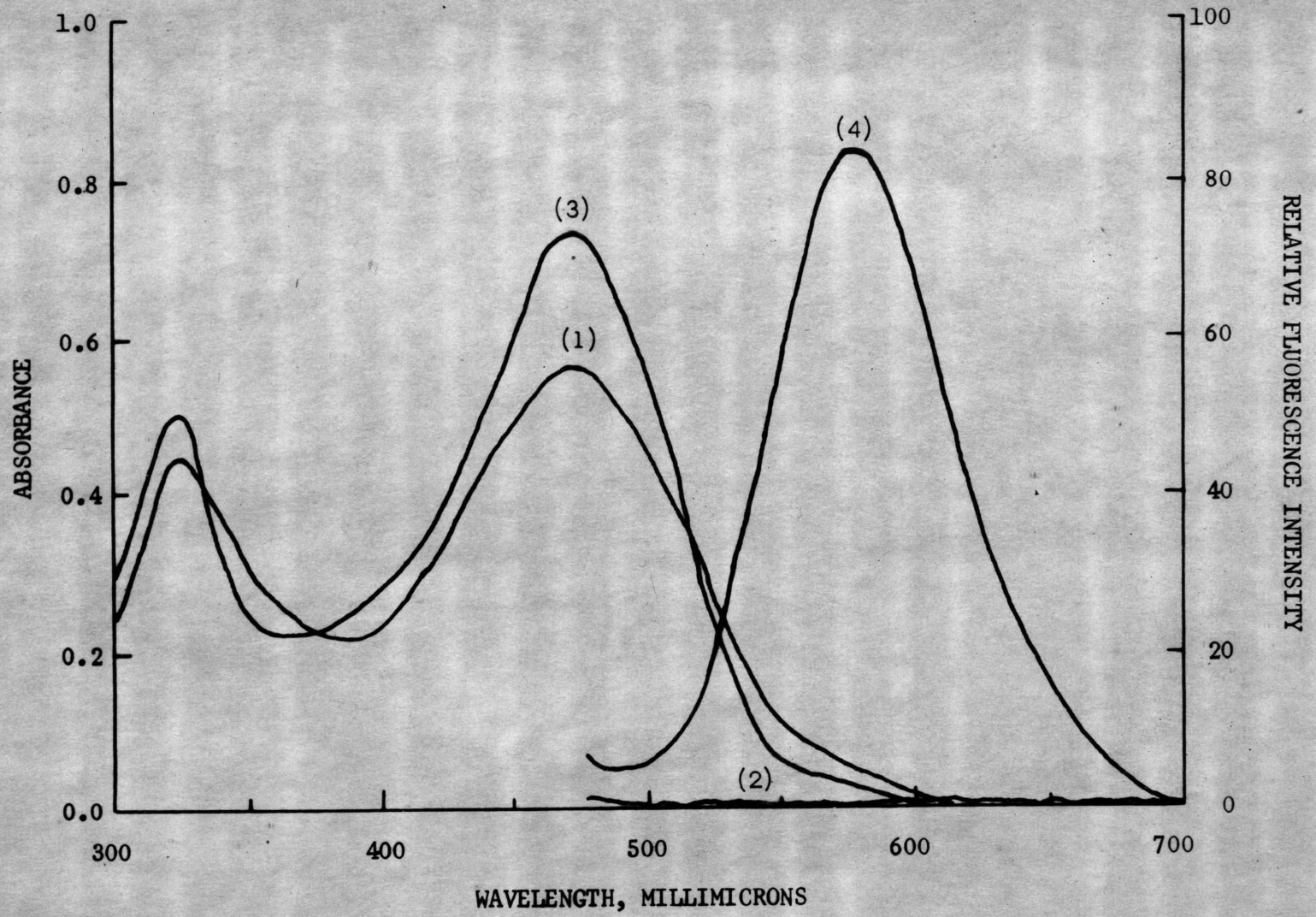

Figure 1. Absorption and fluorescence spectra of 5×10^{-5} M o,o'-dihydroxyazobenzene (1)(2) and 5×10^{-5} M magnesium-o,o'-dihydroxyazobenzene (3)(4) respectively, in ethanol, determined with a Beckman Model DK-2A spectrophotometer.

CS-5-60 and CS-3-67, in excitation and fluorescent beams, respectively. Spectral transmittance characteristics of these two filters are shown in Figure 2. These filters were compatible (i.e. transmission of the primary and secondary filters did not overlap) and their spectral characteristics matched those of the magnesium-o,o'-dihydroxyazobenzene complex.

Factors Affecting the Fluorescence Intensity

The fluorometric method of determining Mg with o,o'-dihydroxyazobenzene may be carried out in different media. Because fluorescence was enhanced by the presence of ethanol, a water-ethanol medium was used. The increase in intensity with increasing concentration of ethanol was sufficiently great that care was necessary to always introduce the same volume of ethanol. This was done expeditiously by introducing the ethanol at the same time as the o,o'-dihydroxyazobenzene and the buffer in the form of a working solution. For convenience, an automatic pipette was used.

Diehl et al. (6) found that for maximum fluorescence, solutions should be buffered near a pH of 10.6.

Magnesium-o,o'-dihydroxyazobenzene appeared to form instantly and it was stable indefinitely.

Fluorescence intensity was temperature-dependent and decreased as the solution temperature increased even slightly above room temperature. Thus, fluorescence of samples was read rapidly to avoid warming in the sample compartment.

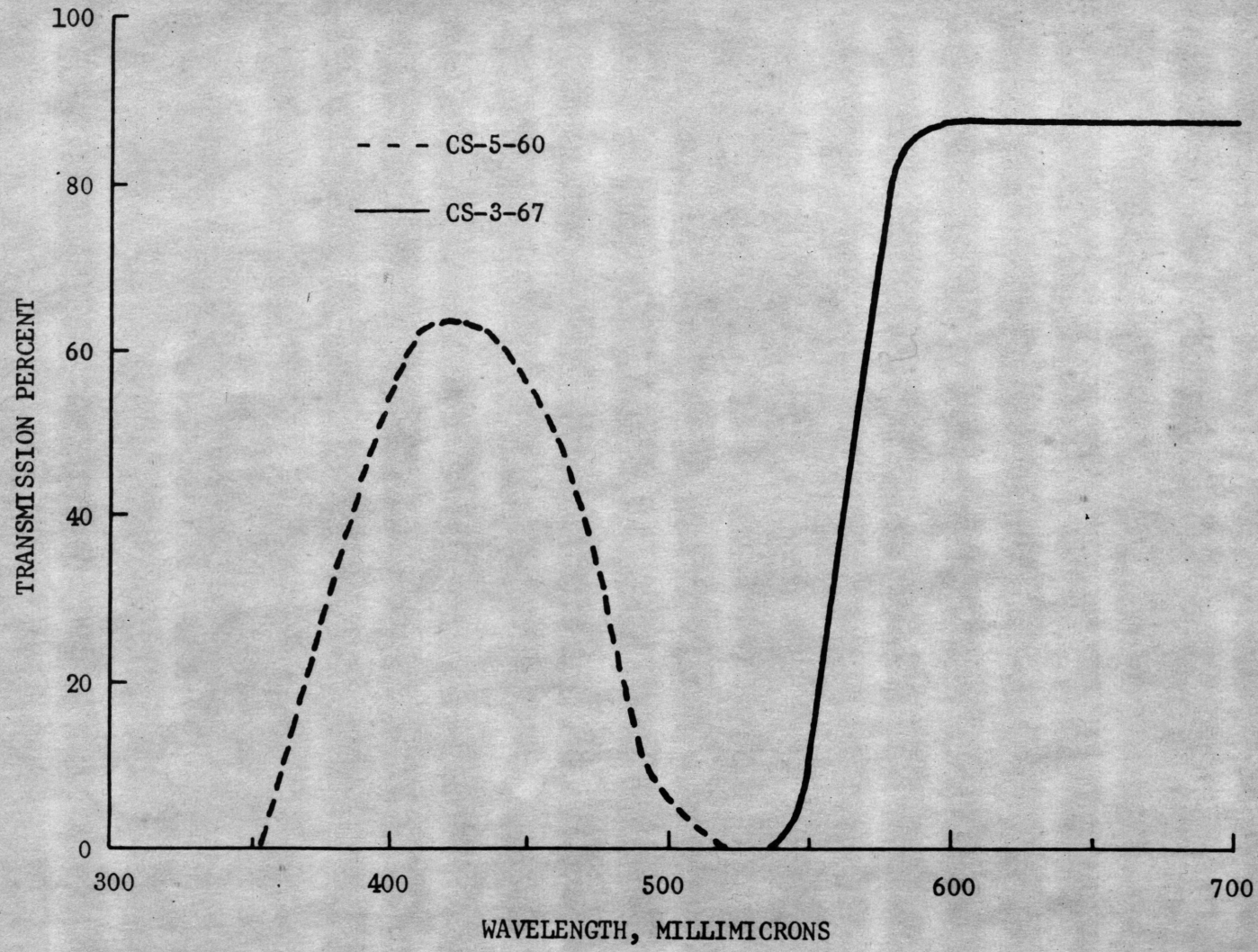

Figure 2. Spectral transmittance characteristics of Corning glass color filters CS-5-60 and CS-3-67 as obtained from Corning glass color filter bulletin CF-3.

General Soil Analysis

Air dried soil samples were characterized with routine laboratory analysis. Results of chemical determinations on eight soil samples are given in Table 1.

The soils were selected on the basis of exchangeable Mg, per cent Mg saturation, and cation exchange capacity. Extremes and random examples of the three properties were selected as test soils for the fluorometric determination of Mg. The soils selected are examples of the Great Soil Groups: Grey-brown Podzolic, Brunizem, Chernozem, and Chestnut.

Working Concentration

To determine the optimum working concentration Mg solutions varying widely in concentration were prepared and fluorescence intensity was measured. The portion of the curve where fluorescence intensity was directly proportional to concentration was selected as the desirable working concentration. Above this concentration, self-absorption occurred, and consequently the proportionality deviated from linearity. The best concentration range selected from Figure 3 was from 0.0 to 2.0×10^{-5} M Mg.

Standard Calibration Curve

A calibration curve prepared from standard Mg solutions is shown in Figure 4. The linear relationship between concentration and

Table 1. Some chemical properties of the soil samples used in this study.

Soil description		pH Saturated paste	Electrical conductivity mmhos/cm.	$CaCO_3$ equiv. %	C.E.C. me./100 g	Exch. Mg me./100 g	% Mg sat.	Exch. Ca me./100 g	% Ca sat.
No.	Series*								
1.	Conover+	6.8	0.95	0.00	7.72	1.83	24	5.53	72
2.	Conover	5.8	0.78	0.16	8.61	.57	7	5.45	63
3.	Zimmerman	5.9	0.18	0.00	6.01	.82	14	3.10	52
4.	Clarion	7.2	0.58	0.50	10.74	2.36	22	8.90	83
5.	Aastad	6.3	0.50	0.10	21.82	7.58	35	11.46	53
6.	Barnes	7.4	0.60	0.58	16.43	3.86	28	14.39	88
7.	Brookings	5.8	0.48	1.00	26.68	12.24‡	46‡	11.87‡	45‡
8.	Morton	6.6	0.55	0.55	23.10	5.71	25	16.51	71

*Series names temporarily assigned to soils sampled for this study.
+Similar to soil number 2 in location and type but applications of dolomitic limestone had been applied to field.
‡These values may have been high due to free alkaline earth carbonates.

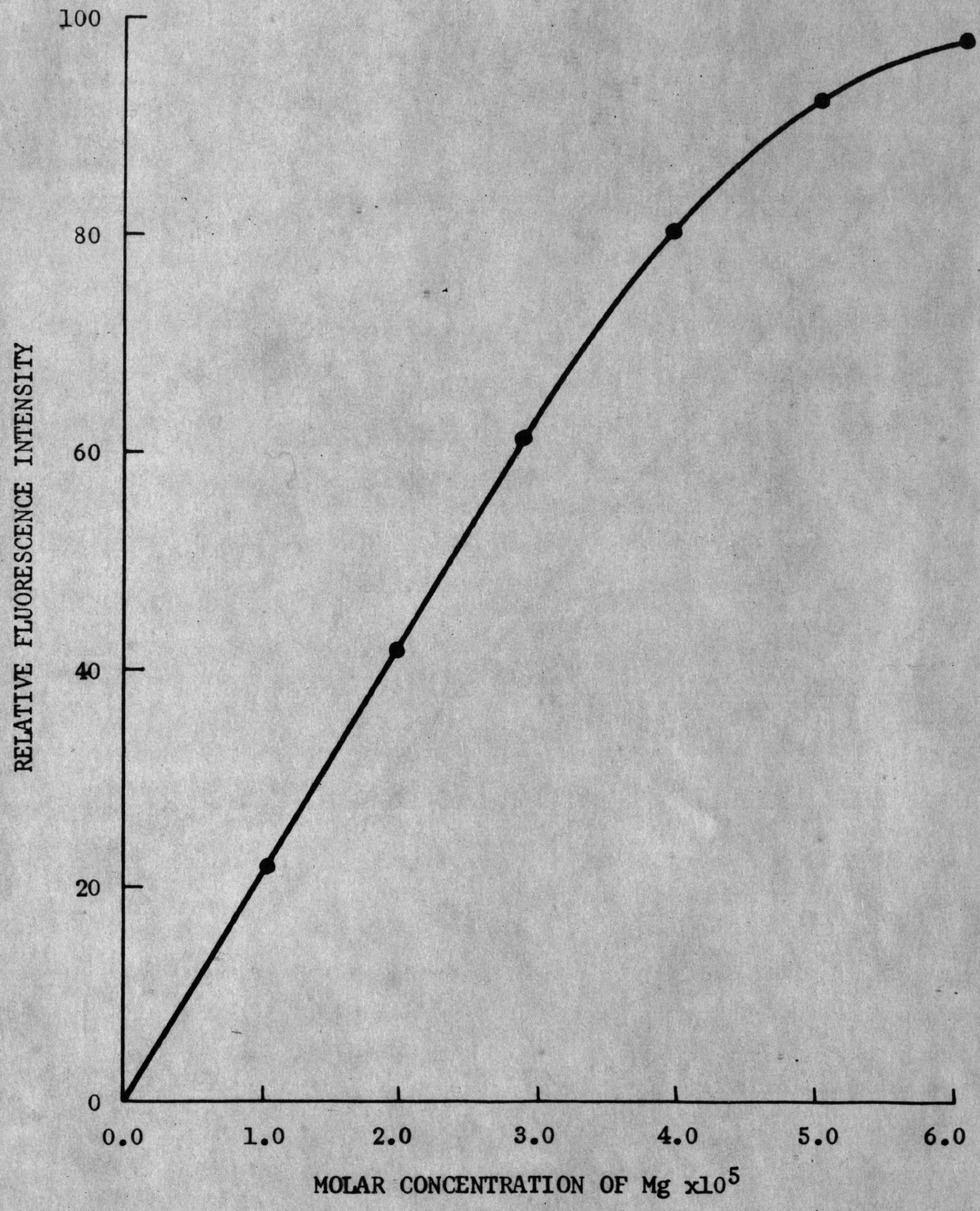

Figure 3. Fluorescence intensity of a wide range of Mg concentrations to determine most desirable working range. Working solution (I) of o,o'-dihydroxyazobenzene was used. Measurements made on a Turner Model 110 fluorometer with the aperture range selector set at 1X.

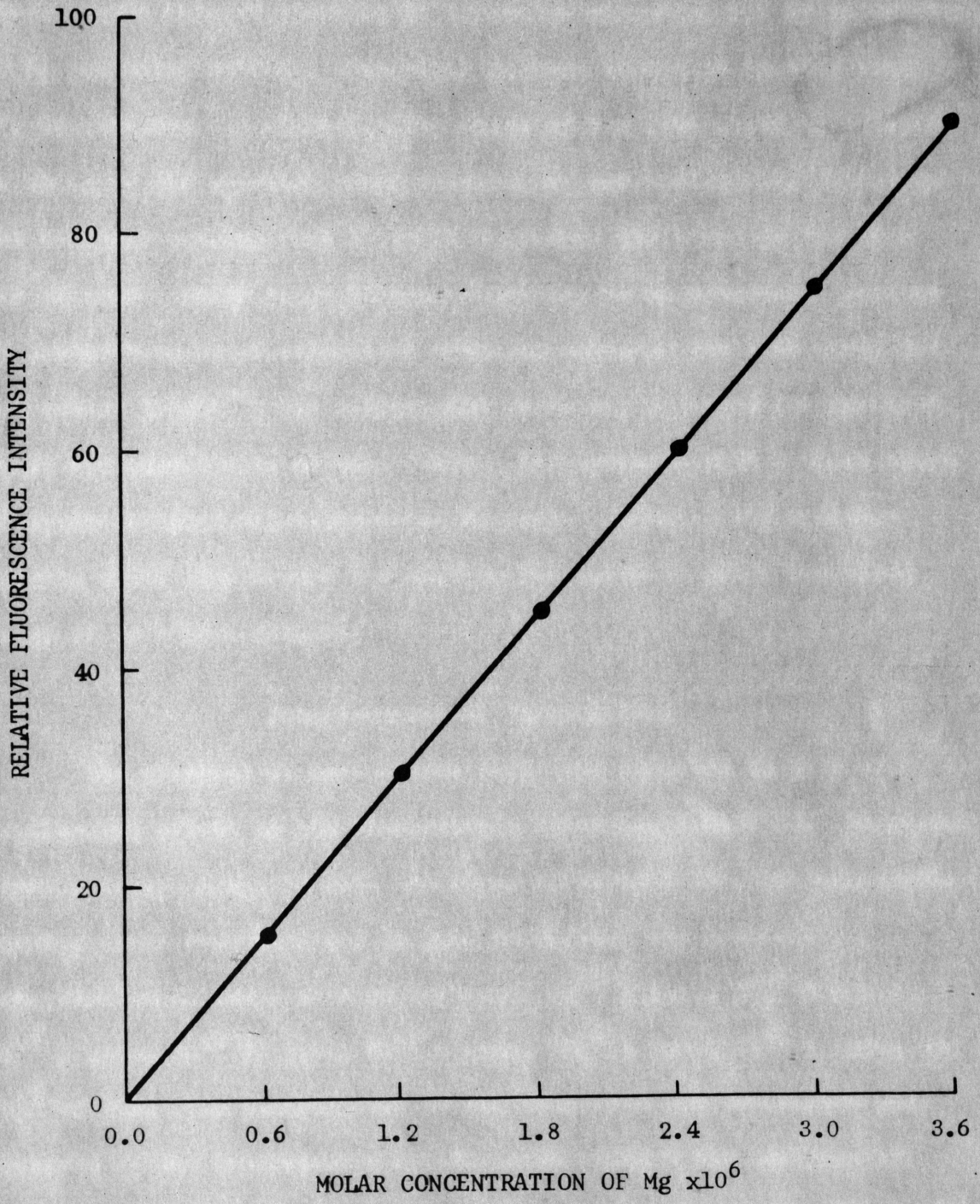

Figure 4. Calibration curve for the fluorometric determination of Mg in soil extracts using working solution (I) of o,o'-dihydroxyazobenzene. Measurements made on a Turner Model 110 fluorometer with the aperture range selector set at 3X.

fluorescence made it possible to determine concentration by calculation or by graphical methods. Graphical methods were used in this study for convenience and clarity. The slope of the line could be adjusted by changing the aperture range selector of the instrument. Changing the aperture opening from 3X to 1X decreased the slope of the line and allowed a wider range of concentrations to be measured. Because Mg concentrations of saturation extracts selected for this study did not exceed 3.6×10^{-6} M the calibration curve was adjusted for greatest slope and sensitivity of that range. For extracts with a wider range of concentrations dilutions could be used. Changing the aperture opening would also change the calibration curve to match the wider range of Mg concentrations.

Interferences of Foreign Ions

The presence of most common soil ions appeared to have no detrimental effect on the fluorometric determination of Mg. Magnesium standards were prepared with additions of several foreign ions approximating their concentration in soil extracts. The ions and concentrations tested were as follows: Al^{+3} 1 ppm., PO_4^{-3} 5 ppm., Mn^{+2} 1 ppm., Na^{+1} 25 ppm., Cu^{+2} 1 ppm., SO_4^{-2} 5 ppm., Cl^{-1} 1 ppm., and NO_3^{-1} 50 ppm. The fluorescence of all standards was determined and compared to the fluorescence of standards without the addition of foreign ions. The results of these experiments showed the effect of foreign ions on the fluorometric determination of Mg to be in most cases, insignificant.

Diehl et al. (6) showed the presence of large amounts of Ca suppressed the fluorescence. This was confirmed in the study reported here. Relative fluorescence of Mg standards containing three concentrations of Ca was recorded in Figure 5. Calcium added to soil extracts appeared to cause a small positive error in the Mg determined. Calcium, because of its similarity to Mg, probably reacted slightly with o,o'-dihydroxyazobenzene and gave a positive interference. The extent of this interference is shown in Table 2.

Table 2. The effect of added Ca on the fluorometric determination of Mg in soil saturation extracts using working solution (I).

	No Ca added	75 ppm. Ca added		150 ppm. Ca added	
Soil No.	Mg found ppm.	Mg found after add. ppm.	Difference ppm.	Mg found after add. ppm.	Difference ppm.
1.	37.5	37.9	+0.4	38.0	+0.5
2.	16.1	16.4	+0.3	16.4	+0.3
3.	5.6	5.6	0.0	6.3	+0.7
4.	18.2	18.4	+0.2	18.4	+0.2
5.	26.9	27.1	+0.2	27.5	+0.6
6.	15.1	15.8	+0.7	15.9	+0.8
7.	24.7	25.1	+0.4	25.2	+0.5
8.	7.8	8.3	+0.5	8.1	+0.3

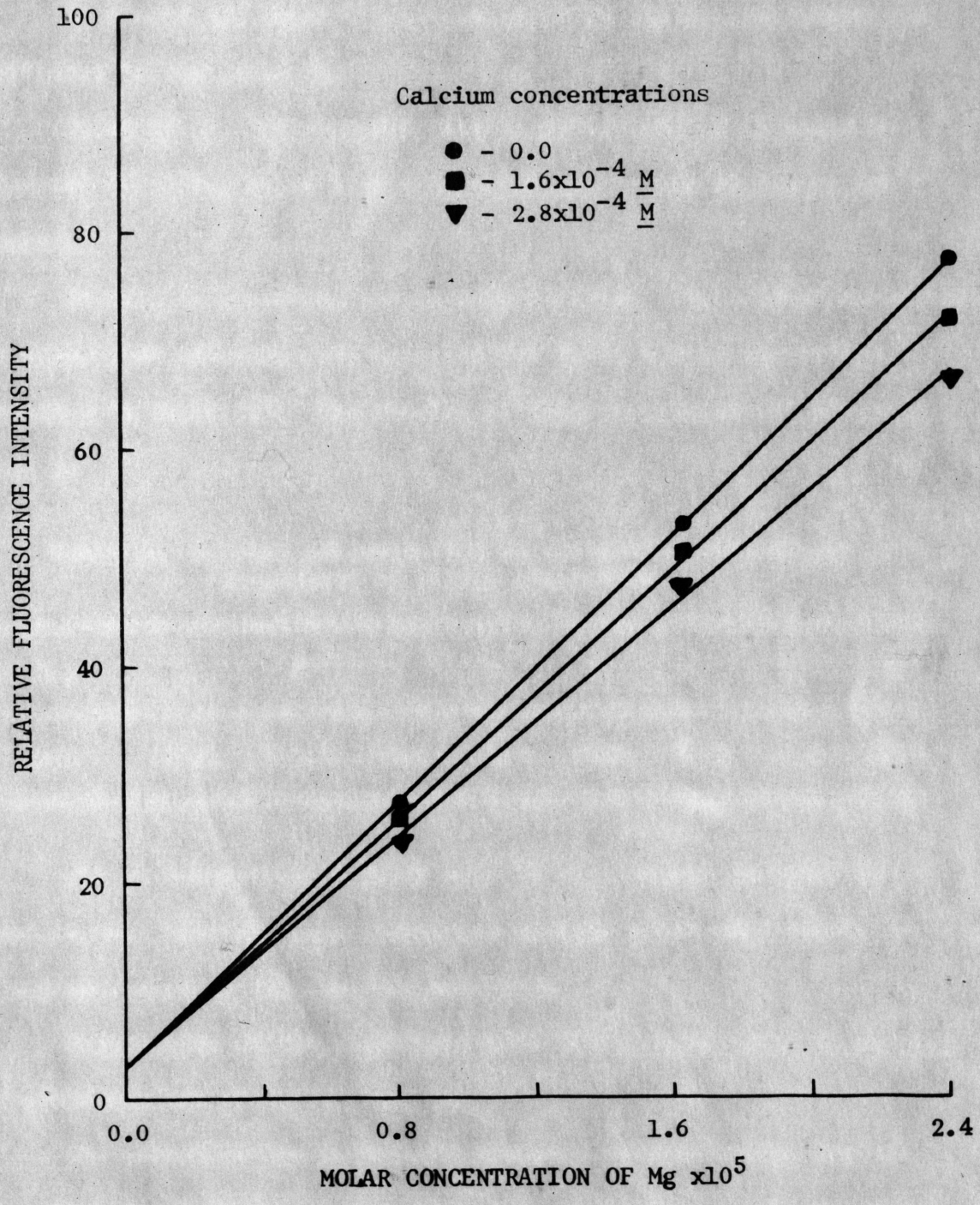

Figure 5. Relationship of fluorescence of Mg standards in presence of three concentrations of Ca using working solution (I) of o,o'-dihydroxyazobenzene. Measurements made on a Turner Model 110 fluorometer with the aperture range selector set at 1X.

In almost all cases a positive error in the Mg determined was attributed to the added Ca.

Diehl et al. (6) reported that large amounts of Ca could be tolerated by adding Ca at high concentrations to the standards and samples.

To help in selecting the Ca concentration which should be added, the saturation extracts were analyzed for Ca by atomic absorption according to David (5). The results of this experiment are shown in Table 3.

Table 3. Calcium concentration in saturation extracts as determined by atomic absorption.

Soil No.	1	2	3	4	5	6	7	8
Ca ppm.	164.1	114.4	19.6	64.2	60.0	76.5	81.0	28.6

The Ca concentration level must be high enough to cover Ca found in soil extracts plus additional amounts which were added. A Ca concentration of 500 ppm. was selected.

The results of the experiment with 500 ppm. Ca added to samples and standards are shown in Table 4.

The errors of the Mg determined were, in most cases, reduced when working solution (II) was used. The difference in errors between Tables 2 and 4 may not be significant, but addition of Ca at high

Table 4. The effect of Ca on the determination of magnesium using working solution (II) with Ca added. Working solution (II) added 500 ppm. Ca to all samples and standards.

	No Ca added	75 ppm. Ca added		150 ppm. Ca added	
Soil No.	Mg found ppm.	Mg found after add. ppm.	Difference ppm.	Mg found after add. ppm.	Difference ppm.
1.	37.9	37.9	+0.0	38.6	+0.7
2.	16.2	16.3	+0.1	16.3	+0.1
3.	6.2	6.4	+0.2	6.4	+0.2
4.	19.3	19.2	-0.1	19.4	+0.1
5.	27.7	27.8	+0.1	28.0	+0.3
6.	9.1	9.1	0.0	9.2	+0.1
7.	24.6	24.8	+0.2	25.0	+0.4
8.	7.9	7.9	0.0	8.2	+0.3

levels indicated Ca interference can be reduced. The errors in Table 4 would be insignificant when compared to other possible errors of volumetric procedures. Therefore, it can be concluded that high levels of Ca added to the samples and standards, in the form of a working solution, are a possible means of reducing Ca interference.

Fluorometric Determination of Magnesium

The results of the fluorometric determination of Mg in saturation extracts of the soils selected from this study are shown in Table 5. The Mg content of the soils selected do not cover as wide a

range as would be expected in all soils, but varied enough for the purpose of this study.

Table 5. Magnesium concentration in saturation extracts as determined by the fluorometric method using working solution (I).

Soil No.	1	2	3	4	5	6	7	8
Mg ppm.	37.9	17.3	4.9	14.0	21.0	12.8	20.1	6.0

All samples were prepared and analyzed in duplicate. The results shown are averages of the duplicates. Some difficulty was found in reproducing results with saturation extracts. This would be expected because of the problem of always adding the same amount of water and removing the same volume of extract. Reproducibility of the Mg estimation in any given extract was excellent in all cases. Duplicate determinations of any one sample did not differ by more than 1%.

Magnesium was also determined in ammonium acetate extracts. Results of the fluorometric determination of exchangeable Mg in ammonium acetate extracts are shown in Table 6.

Table 6. Exchangeable Mg in ammonium acetate extracts as determined fluorometrically using working solution (I).

Soil No.	1	2	3	4	5	6	7	8
me.Mg/100 g	1.72	.90	.97	2.70	7.24	3.54	11.10	5.24

The results shown are averages of two determinations on each soil. In most cases, there was very good agreement between duplicate samples. The small amount of ammonium acetate with which the Mg was transferred appeared to have no effect on the fluorescence intensity. To test this, standards were prepared with 0.100 ml. ammonium acetate included and fluorescence measured. The fluorescence of these standards did not differ from standards prepared without ammonium acetate added.

Recovery Experiments

Also tested was the ability of the fluorometric procedure to detect and quantitatively measure known amounts of Mg which had been added to soil extracts. These observations are recorded in Table 7.

Table 7. Recovery of Mg added to saturation extracts of soils by fluorescence analysis using working solution (I).

		5 ppm. Mg added		10 ppm. Mg added	
Soil No.	Mg initially found ppm.	Mg found after add. ppm.	Recovery %	Mg found after add. ppm.	Recovery %
2.	10.1	15.6	103	20.8	104
3.	4.7	9.9	102	15.2	103
4.	15.4	20.3	100	25.3	100
5.	22.8	27.5	99	33.8	103
6.	11.3	16.1	98	21.8	102
7.	19.7	24.3	98	30.2	102
8.	9.6	14.6	100	19.4	99

Results shown include data for soils 2 through 8. Soil number 1 was not available at the time this experiment was completed.

One very desirable quality of fluorescence analysis is its ability to measure very small quantities of material. Results of these experiments showed that the fluorometric procedure accurately determined small amounts of Mg. The two levels of Mg added to the extracts were 5 and 10 ppm. In all cases, the added Mg was determined accurately by the fluorometric method. The results showed all recoveries were within 5% error.

Comparison with Other Methods

Saturation extract Mg concentrations by the fluorometric method are compared with the atomic absorption and thiazol yellow colorimetric results in Table 8.

All samples were prepared in duplicate. The results shown are averages of two determinations in all cases. All samples showed very good duplication with the fluorometric method. The fluorometric method duplicated Mg concentrations in extracts as well as the atomic absorption method, and even better in some cases. Duplicates of the thiazol yellow procedure did not agree as well. Some values differed by more than 5%.

In most cases, there was better agreement between the fluorometric and atomic absorption methods than between these two methods and the thiazol yellow colorimetric method. More confidence was

Table 8. Comparison of fluorometric analysis using working solution (I), atomic absorption, and thiazol yellow colorimetry for estimating Mg concentrations in saturation extracts of eight soils.

Soil No.	Fluorometric ppm.	Atomic Absorption ppm.	Thiazol Yellow ppm.
1.	37.9	38.9	32.0
2.	17.3	17.9	16.8
3.	4.9	4.9	1.1
4.	14.0	14.0	13.4
5.	21.0	21.4	21.1
6.	12.8	20.4	21.3
7.	20.1	20.4	21.3
8.	6.0	5.8	7.5

placed in the atomic absorption method for comparison because of problems of color stability and duplication encountered with the thiazol yellow colorimetric method. In all cases, agreement between the fluorometric and atomic absorption method was well within 5%.

Exchangeable Mg determined with the fluorometric method is compared with results obtained by the atomic absorption method in Table 9.

The results shown are averages of two determinations in all cases. Good agreement was obtained in most cases. The fluorometric

Table 9. Exchangeable Mg in ammonium acetate extracts as determined fluorometrically using working solution (I) and with atomic absorption.

Soil No.	Fluorometrically me.Mg/100 g	Atomic Absorption me.Mg/100 g
1.	1.72	1.83
2.	.90	.57
3.	.97	.82
4.	2.70	2.36
5.	7.24	7.58
6.	3.54	3.86
7.	11.10	12.24
8.	5.24	5.71

method appeared to be very well qualified for determination of exchangeable Mg in ammonium acetate extracts.

SUMMARY AND CONCLUSIONS

Magnesium concentrations in ammonium acetate and saturation extracts of soils were determined successfully by fluorescence with o,o'-dihydroxyazobenzene. The procedure was tested for foreign ion interference. Recovery experiments were used to test sensitivity. Magnesium concentrations determined in extracts from eight soils were compared with results obtained by two other analytical methods, atomic absorption and thiazol yellow colorimetry.

The fluorometric method proved adaptable to determination of Mg in ammonium acetate and saturation extracts. Most foreign ions did not significantly interfere. Calcium caused a small positive error but when high levels of Ca were added to both standards and samples, this error was reduced to insignificance. Recovery experiments showed the procedure was very sensitive and accurately measured small quantities of Mg. Comparison studies showed the fluorometric method determined Mg as accurately as atomic absorption and more precisely than the thiazol yellow method. The procedure was rapid, convenient, and sensitive enough for most analytical applications.

The fluorometric determination of Mg using the usual reagent produced good results with soils used in this study. However, it would be recommended that the working solution containing Ca be used for soils with widely varying concentrations of Ca.

Simplicity of fluorescence analysis allows the use of relatively simple instruments to make fluorescence measurements. The Turner

Model 110 fluorometer used in this study is quite economical and simple to operate. Because many soil laboratories do not employ fluorometers, the application of other instruments is desirable. The Beckman Model DU spectrophotometer commonly used in soils laboratories can easily be converted for measurements of fluorescence. Equipment necessary for the conversion includes a Beckman Model No. 73500 fluorescence attachment, a Beckman F4T5/BLUE lamp, and a Corning CS-5-60 glass color filter. The spectrophotometer must also be equipped with a photomultiplier attachment. With these changes, the fluorometric determination of Mg presented in this study could be adapted for use with the Beckman Model DU.

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